Recent researches have now shown that the **relative concentration of auxin** on two sides of the abscission layer has **regulatory influence** on the production of **ethylene** that stimulates leaf abscission. At the time of abscission, concentration of **auxin** in the laminar region **decreases** with simultaneous **increase in ethylene production**. This also **increases sensitivity of cells of abscission zone to ethylene** which now synthesize cell wall degrading enzymes such as **cellulases** and **pectinases**. Activity of these enzymes results in cell wall loosening and cells separation ultimately leading to leaf abscission.

(Although ABA is certainly involved in **senescence** of leaves but it is **not concerned in the abscission of leaves**. It is probable that through its promotion of senescence, ABA might increase production of **ethylene** that may stimulate abscission).

Q. What is fruit ripening ? Give a brief and critical account of physiology of fruit ripening mentioning clearly the distinction between climacteric and non-climacteric fruits, role of ethylene and other phytohormones in ripening, and changes in structure and composition responsible for symptoms of fruit ripening. Add brief paragraphs on external environmental control of fruit ripening.

FRUIT RIPENING

Introduction

The process of fruit ripening is intimately associated with phenomenon of **senescence**. Senescence of a plant organ is usually defined as final stage in its growth and development (*i.e.*, ontogeny) during which a series of essentially irreversible or **deteriorative events** occur leading to cellular breakdown and death. **Fruit ripening** on the other hand, refers to **changes occurring during early stages of senescence of fruits** which make them **fit for consumption** (or **acceptable to eat**). Such changes typically include **change in colour, texture, taste** and **flavour** (**aroma**) of the fruit.

From botanical point of view, fruit ripening means that the **seeds are ready for dispersal** and the attractive colours, sweet or tasty juicy pulp and characteristic aroma of the ripened fleshy fruit might be related to this function. In case of seeds whose dispersal depends on ingestion by animals, **fruit ripening** is in fact **synonymous with edibility**. But in **dry fruits**, where the seeds require mechanical or other means for dispersal, **fruit ripening** may be considered as **drying followed by splitting**.

Because of commercial importance of edible fruits in agriculture and horticulture, most studies on fruit ripening have been done on **edible fruits**.

Fruit ripening may occur while the fruit is still attached to plant (as is usual in non-climacteric fruits) or after their harvest (as in climacteric fruits).

If not consumed in time, the ripened fruits begin to rot due to invasion by saprophytic organisms.

Climacteric and Non-climacteric Fruits and Role of Ethylene in Fruit Ripening

In 1920s, **Kidd & West** (1925) were the first to show that **onset of the visible ripening changes** in apples was marked by **dramatic increase in the rate of respiration** and they coined the word **respiration climacteric** to describe this critical phase in the life of the fruit. However, it is now known that respiration climacteric is exhibited by certain fruits only and not by all types of the fruits.

The role of **olefinic gas ethylene** in promoting ripening of fruits is known to scientists for about a century. In many cases, treatment of unripe fruits with ethylene **hastens ripening** with dramatic increase in production of ethylene during initiation of ripening. But, not all fruits respond to ethylene treatment. However, all **those fruits which ripen in response to ethylene treatment also exhibit respiration climacteric and are called as climacteric fruits** such as

apple, banana, tomato, mango etc. On the other hand, **those fruits which do not respond to ethylene treatment, neither show respiration climacteric nor they exhibit significant increase in ethylene production and are called as non-climacteric fruits**. Examples are **citrus fruits, grapes, strawberry** etc.

A list of some common climacteric and non-climacteric fruits is given in Table 17.1.

Table 17.1. Some commonly known climacteric and non-climacteric fruits.

Climacteric Fruits	Non-climacteric Fruits
Apple	*Capsicum annum* (Pepper)
Apricot	Citrus fruits :
Avocado (*Persea americana*)	Lemons
Banana	Mandarins
Cantaloupe (Musk-melon)	Oranges
Cherimoya (*Annona* spp.)	Grapes
Feijoa (Family Myrtaceae)	Pineapple
Fig	Snapbean
Mango	Strawberry
Melon	Watermelon
Olive	
Peach, Pear	
Persimmon (*Diospyros* spp.)	
Plum	
Tomato	

- Depending upon the climacteric fruit involved, the peak in ethylene production can precede (*e.g.*, banana), coincide (*e.g.*, mango, pear, avocado) or follow (*e.g.*, apple, cherimoya, tomato) the peak in respiration climacteric.
- **Kidd & West** (1945) have introduced the concept of **threshold concentration of ethylene** which must accumulate in the tissue in order to initiate the ripening response.
- It had been found that for many ethylene dependent responses in plants, the threshold value was about 0.1 ppm and that conc. of 1 to 10 ppm was saturating. The levels of ethylene that ultimately accumulate in fruits can be very much in excess of this minimum level (sometimes even ten thousand times greater) required to elicit respiration climacteric and ripening.
- However, once the climacteric has been initiated, further treatment with exogenous ethylene has no effect in promoting ripening processes.
- In climacteric fruits, increased ethylene production as a result of external ethylene treatment is believed to be **autocatalytic**.
- Whether the fruit is climacteric or non-climacteric *i.e.*, it responds to ethylene treatment or not, **a minimum threshold level of endogenous ethylene is necessary for ripening of all types of fruits**. This has unequivocally been proved by experiments with **transgenic plants** such as transgenic tomatoes. By making expression of antisense version of **ACC Synthase or ACC Oxidase** (*i.e.*, by blocking the biosynthesis of ethylene) in such plants, ripening of tomatoes was completely inhibited which could be restored by treatment with externally applied ethylene only. These experiments have also opened new vistas in manipulating (*i.e.*, delaying or hastening) fruit ripening through biotechnology.

Hormonal Control of Fruit Ripening

The control of maturation and initiation of fruit ripening is believed to be due to **interaction and balance between promotory and inhibitory effects of different phytohormones. Ethylene** is one promoting factor, **abscisic acid** is another. The role of ethylene in fruit ripening has already been discussed earlier. The role of other phytohormones in ripening is briefly discussed below :–

Abscisic acid (ABA)

ABA plays an important regulatory role in fruit ripening. There is marked accumulation of ABA in fruit tissues during ripening. In **climacteric fruits** such as **avocado** and **pear**, the level of ABA is constant during maturation but rises rapidly during ripening and coincides with rise in ethylene production during ripening. **Adato** *et al* (1976) have shown threefold increase in free ABA level during ripening of detached avocado pear at 19°C to a maximum level of about 7000 µg/kg fresh weight (Fig. 17.43).

Even in **non-climacteric fruits** such as **citrus fruits** and **grapes** where there is no rise in ethylene production during ripening, the ABA level increases markedly. Application of ABA to mature fruits is known to enhance ripening processes.

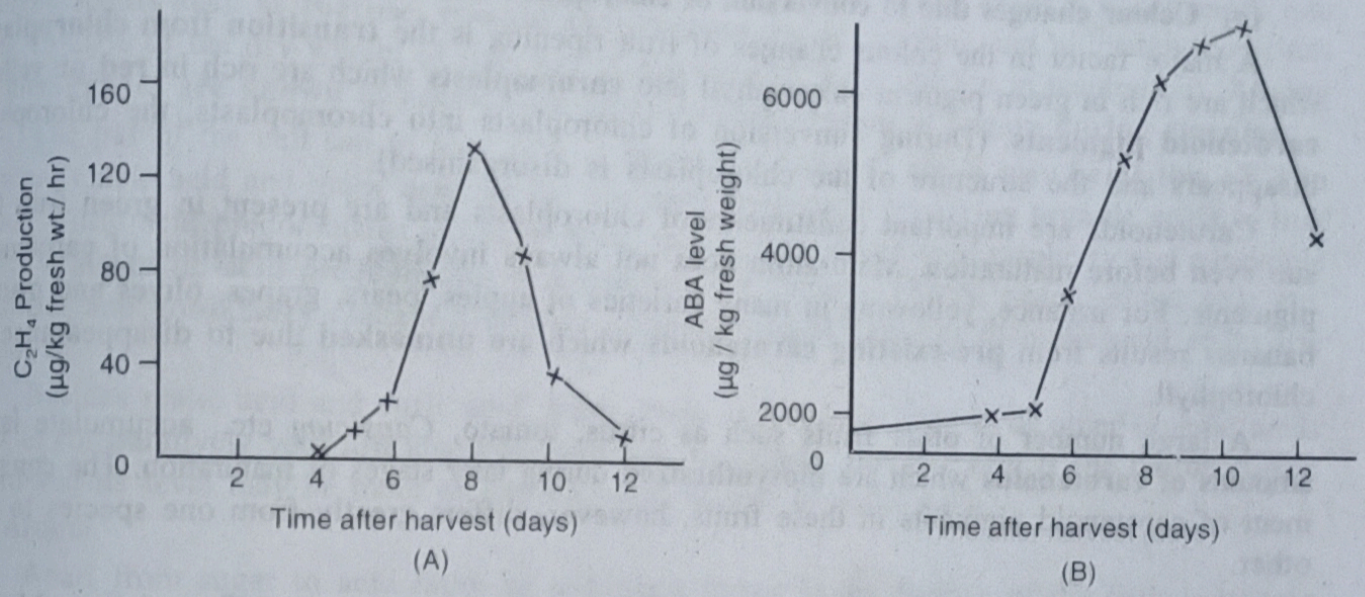

Fig. 17.43. Changes in level of (A) ethylene production and (B) free ABA during ripening of avocado fruits (After Adato *et al*, 1976).

Auxins

Indole-acetic-acid (IAA) is probably an endogenous hormonal inhibitor of ripening. It acts both as an **inhibitor of ripening** and at the same time **promotor of ethylene biosynthesis**.

Conflicting results have been obtained with **synthetic auxins** on fruit ripening. For instance, certain synthetic auxins such as **2, 4, 5-trichlorophenoxy propionic acid** and **2, 4, 5-trichlorophenoxy acetic acid** are known to improve anthocyanin colouration of apples along with other ripening processes. Whereas, **2, 4-dichlorophenoxy acetic acid** delays yellowing of lemons and is used commercially to delay ripening of citrus fruits after harvest.

Gibberellins

Gibberellins are also known to delay fruit ripening in plants. Gibberellins interfere with degradation of chlorophyll and biosynthesis of carotenoids and anthocyanins. Application of GA_3 in concentrations as low as 0.1 mg/l effectively delays degreening of detached citrus fruits. Gibberellins are also known to promote regreening of Valencia oranges.

Cytokinins

The role of cytokinins in delaying senescence in plants is well known and this effect of cytokinins has also been obtained in delaying ripening processes of fruits especially those related to chloroplasts (*i.e.*, degreening). However, olives are exceptions where cytokinins promote accumulation of anthocyanins in the fruit.

Symptoms of Fruit Ripening

1. Texture (Softening of Fruit)

The changes in the texture of fruit during ripening result due to changes in the structure and composition of their cell walls.

During ripening of fruit, there is **extensive degradation of cell walls** due to increased activities of cell wall degrading enzymes such as **cellulases** and **pectinases** etc. resulting in softening of the fruit.

2. Colour

The factors responsible for changes in colour of fruit during ripening may be due to changes in pigments localised **in chloroplasts** or those which are stored **outside chloroplasts in vacuoles**.

(*a*) Colour changes due to conversion of chloroplasts into chromoplasts – The carotenoids.

A major factor in the colour changes of fruit ripening is the **transition** from **chloroplasts** which are rich in green pigment **chlorophyll** into **chromoplasts** which are rich in **red or yellow carotenoid pigments**. (During conversion of chloroplasts into chromoplasts, the chlorophyll disappears and the structure of the chloroplasts is disorganised).

Carotenoids are important constituents of chloroplasts and are present in green fruit tissue even before maturation. Maturation does not always involves accumulation of carotenoid pigments. For instance, yellowing in many varieties of apples, pears, grapes, olives and mature bananas results from **pre-existing carotenoids** which are **unmasked** due to disappearance of chlorophyll.

A large number of other fruits such as citrus, tomato, *Capsicum* etc., accumulate large amounts of **carotenoids** which are **biosynthesized** during later stages of maturation. The complement of carotenoid pigments in these fruits, however, differs greatly from one species to another.

In tomatoes, the carotenoid pigments are dominated by **lycopene** and **β-carotene**. Mature citrus fruits contain **over 115 different carotenoids** (about 1/3 of the total carotenoids occurring in nature). Besides 40-C carotenoids, the citrus peel also contains 30-C carotenoids such as **β-citraurin** which is responsible for bright **orange and red colour of oranges and tangerines**. In oranges, besides increase in **xanthophyll conc.**, there is also an increase in their **esterification**. Upto 60% esterification of the xanthophylls has been reported by scientists in orange peel.

(*b*) Colour changes due to pigments stored outside chloroplasts (i.e., in vacuole) – The anthocyanins.

Anthocyanins are **water soluble phenolic pigments** which accumulate in **vacuole** and impart **red, blue** and **purple colours** to many fruits such as ripening fruits of apple, grape, strawberry etc. Anthocyanins exist as complex conjugates of parent **aglycones** called as **anthocyanidins**. There are **six main anthocyanidins** which occur in fruits as **3-glycosides**. These are, **pelargonidin, cyanidin, peonidin, delphinidin, petunidin** and **malvidin**.

Cyanidin -3-galactoside is the chief pigment responsible for the colour of **red apple varieties**. **Pelargonidin-3-glucoside** is the chief pigment of **ripe strawberries**. In some fruits such

as **grapes, acylated anthocyanins** are found. Frequently, many different anthocyanins are present in the same tissue each contributing to the colour of the fruit. (For details of anthocyanins, see Chapter 24).

3. **Taste**

Generally, there is **decrease in acidity** and **increase in sweetness** during fruit ripening. Some fruits like bananas are however, exceptional, where acidity actually increases (from pH 5.4 to 4.5) during ripening due to increase in content of organic acids such as **malic acid** and **citric acid**.

In fruits such as **melons** (which have essentially no reserve carbohydrates), there is no increase in sugar content during ripening after harvest although it does increase during ripening when the fruit is attached to parent plant because of transport from leaves. In most fruits, however, **starch** occurs as chief **carbohydrate reserve** which is **converted into sugars** to impart **sweetness** to the ripe fruit.

The **absolute levels of sugars and acids and** also the **ratio of sugars to acids**, play an important role in taste of ripe fruit. In many fruits, **disappearance of phenolic compounds including tannins** during ripening also contributes to characteristic taste of the fruit.

Unripe green fruits of banana contain 20-25% starch and almost all of it is converted into simple sugars such as **sucrose, glucose** and **fructose** during ripening and ultimately constituting 15-20% of the dry weight of ripe fruit (some amount being utilized in respiration). **Among fruits, grapes are known to accumulate highest concentration of sugars during ripening.**

The **pH** of the cell sap of fruit cells is frequently **below 7** and it may be **as low as 3 in lemon. Citric acid** and **malic acid** are the two **most frequently occurring organic acids** in fruit cells. Citric acid predominates in Citrus fruits, guava, figs, strawberry, raspberry and pineapple etc., while malic acid predominates in apple, apricot, banana, cherry, peach, plum, pear etc. Tomato and gooseberry contain a mixture of almost equal amounts of malic acid and citric acid.

Besides malic acid and citric acid, many fruits also store a number of other organic acids but in comparatively very low amounts. However, **in grapes, tartaric acid is the major stored acid** and its level may be more or less the same as that of malic acid.

4. **Aroma**

Apart from sugar to acid ratio, an important factor in the flavour of the fruit is **aroma** which arises from the production of **volatile compounds** by the fruit during ripening. These volatile compounds include many different classes of organic compounds such as **organic acids, alcohols, esters, carbonyl compounds, lactones, hydrocarbons, terpenoids** etc.

The contribution of a particular volatile compound to aroma of the fruit depends upon, **(i) the quantity of the compound produced, (ii) the quality of aroma of each compound** and **(iii) sensitivity of the olfactory epithelium tissue of nose** to a range of concs. of that compound.

The quantity of total volatiles produced by fruit is typically from **1 to 20 ppm**, but in certain varieties of bananas it may be upto 300 ppm. In banana, over 200 volatile compounds have been detected each present at below 1 ppm and in some cases only at one part per thousand million. **A volatile compound although produced in very low amounts, may contribute to aroma of ripe fruit if its olfactory threshold level is very low.**

There are two major categories of precursors of volatile compounds, **(i)** the long chain **amino acids leucine, isoleucine** and **valine** and **(ii) the unsaturated fatty acids, linoleic acid** and **linolenic acid**.

Enviromental Control of Fruit Ripening

Environmental factors such as light, temperature, gaseous composition of atmosphere (O_2

and CO_2), and atmospheric pressure have controlling influence on ripening processes and their uses have important implications in storage of fresh fruits prior to marketing.

1. Temperature

The process of ripening occurs in a **relatively narrow range of temperatures** only. In many fruits of tropical and subtropical origin, fruit ripening is inhibited below a certain critical temperature. For instance, in bananas and tomatoes, this critical temp. is in between 10–13°C whereas in certain temperate fruits such as Cox's orange Pippin variety of apples, it may be as low as 3°C.

Besides critical low temp., there is also an upper temp. limit above which fruits fail to ripen properly. **Biale and Young** (1971) have shown that at temperatures above 25°C, the extent of respiration climacteric in avocado pears decreased markedly. Degreening of tomatoes is inhibited in storage at temperatures above 30°C and bananas fail to ripen properly beyond 30–35°C and their pulp becomes soft and watery.

2. Light

Light also has controlling influence on ripening especially **degreening or colouration** of fruit. **Jen (1974)** has observed loss of chlorophyll by red light in detached tomatoes. Other workers have shown that accumulation of the pigment **lycopene** in tomatoes could be induced by red light and reversed by far-red light. Citrus fruits wrapped in **black polythene** show low levels of chlorophylls and carotenoids in their peels, probably due to poor development of chloroplasts in dark.

In apples, grapes and other fruits, exposure to light is essential for biosynthesis of anthocyanins. (Anthocyanin biosynthesis in apple skin was one of the first systems to demonstrate red $\rightleftharpoons$ far-red reversibility of the pigment phytochrome).

Light intensity is also important in degreening process. **Winkler et al (1974)** have shown that over 54% of sunlight intensity was sufficient for full development of colour in grapes. With decrease in sunlight intensity, there was proportional decrease in anthocyanin levels, so much so that in complete darkness the anthocyanins were completely absent.

3. Gaseous composition of atmosphere

(*i*) **O_2 Tension**

Low levels of O_2 (between 1–5%) in the atmosphere are known to delay ripening in number of different fruits.

It has been shown by many scientists that inhibition of ripening at low levels of O_2 in the atmosphere is chiefly because of its effect on involvement of ethylene in initiation of ripening. The biosynthesis of ethylene from methionine is an aerobic process which is completely inhibited in absence of O_2. However, inhibition of ripening due to low levels of O_2 can be reversed by inclusion of some ethylene in low O_2 atmosphere.

(*ii*) **CO_2 Tension**

An increase in atmospheric CO_2 from 3–10% around some fruits delays onset of climacteric and ripening. However, in some fruits such as apples, high conc. of CO_2 may lead to physiological disorders.

The effect of CO_2 in delaying ripening has been related to its effect on action of ethylene. CO_2 is known to act as competitive inhibitor of ethylene action. According to **Burg** (1965), the relative affinity of active site for ethylene and CO_2 is 100000 : 1.

4. Atmospheric Pressure

Burg and Burg (1966) have shown complete inhibition of ripening of banana fruits stored at one fifth of the normal atmospheric pressure in pure O_2 (to maintain atm. O_2 tension). This

inhibitory effect of low atmosphere could be reversed by inclusion of small amount of ethylene in the atmosphere. According to them, reduced atmospheric pressure caused an **increase in diffusivity of ethylene gas** so that its internal concentration was decreased resulting in inhibition of ripening. Addition of **ethylene** in atmosphere made good the deficiency of the latter so that inhibition of ripening was overcome or reversed.

Q. What is totipotency ? Elucidate it in relation to tissue culture studies in plants. Add brief paragraphs on androgenesis and protoplasts culture.

TOTIPOTENCY

It is now generally considered that every living cell in a plant possesses the potentially of producing an entire new plant or in other words it is **totipotent.** This phenomenon of capability of such a totipotent cell in generating an entire new plant is called as **totipotency**.

Tissue culture studies in higher plants have provided most convincing evidence of the existence of totipotency. Cultures of tissues from pith, cortex, cambium, leaf parenchyama and storage parenchyama in a suitable aseptic (sterile) medium grow readily by cell division and cell enlargement to produce a mass of undifferentiated cells called as **callus**. The individual cells isolated from the callus tissue cultures under proper environmental conditions can be shown to behave like zygotes and to produce entire new plants. Therefore, such totipotent cells have capability in **recapitulating the embryonic stages of development** with the eventual formation of mature plants. The culture medium usually consists of inorganic salts, sucrose, vitamins and an organic supplement such as yeast extract, malt extract or coconut milk.

Experiments with callus tissue cultures in showing totipotency of cells have been more successfully carried out in some plants of the families **Umbelliferae** and **Solanaceae** such as carrot, tobacco etc., than in plants of other species. Most significant results in this direction have been obtained by **Steward et al** in their extensive experiments and studies with the growth of carrot root tissue cultures. Small segments of tissues ('explants') from sec. phloem of carrot were taken and grown on a suitable sterile medium. The individual cells were isolated from the carrot root callus tissue by 'washing technique' and cultured. Many of these cells began to behave like zygotes and produced cell masses ('**embryoids**') that resembled various stages of development of embryos. These continued to grow producing roots and shoots and ultimately developed into normally appearing carrot plants. Steward considered the sec. phloem cells of carrot root as totipotent for which two things were essential, (*i*) each individual cell has to be completely freed (separated) from the neighbours and (*ii*) proper environment such as that found in embryo-sac was needed. Coconut milk provides such an environment (coconut milk is a complex mixture of carbohydrates, cyclitols, amino acids, gibberellins, cytokinins, phenolics and other substances).

The tissue culture techniques such as that mentioned above have been successful in growing '**test tube plants**' and also in cultivation of many plants such as orchids, fruit trees etc., through embryoids formation, so much so that '**germ plasm banks**' have now been established in laboratories in many countries all over the world. The main advantage with such an idea is that callus tissue cultures can be maintained for long periods of time and that too on and space. When embryoids are needed for plantlets production, these cul-